

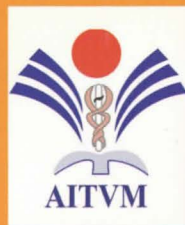
# Proceedings

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Does control  
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## **RECOMBINANT CAPRIPOXVIRUSES EXPRESSING PROTEINS OF BLUETONGUE VIRUS: EVALUATION OF IMMUNE RESPONSE AND PROTECTION IN SMALL RUMINANTS**

PERRIN A.<sup>1</sup>, ALBINA E.<sup>1</sup>, BREAD E.<sup>2</sup>, SAILLEAU C.<sup>2</sup>, PROME S.<sup>1</sup>,  
GRILLET C.<sup>1</sup>, KWIATEK O.<sup>1</sup>, RUSSO P.<sup>3</sup>, THIERY R.<sup>3</sup>, ZIENTARA S.<sup>2</sup>,  
CETRE-SOSSAH C.<sup>1\*</sup>

- 1. Cirad, Control of Emerging and Exotic Animal Diseases Research Unit,  
Campus international de Baillarguet, 34398 Montpellier Cedex 5, France*
- 2. AFSSA-INRA-ENVA, UMR 1161, 23 avenue du Général de Gaulle, 94704  
Maisons-Alfort Cedex, France*
- 3. AFSSA-Unité pathologie des petits ruminants, 105 route des chappes,  
BP111, 06902 Sophia Antipolis, France*

### **ABSTRACT**

Bluetongue is an infectious arthropod-borne viral disease affecting sheep, domestic and wild ruminants caused by Bluetongue Virus (BTV) and transmitted by few species of *Culicoides* (Diptera: Ceratopogonidae). Since 1998, outbreaks of BT involving 6 distinct serotypes have occurred in the Mediterranean basin. The only BTV vaccines currently available are serotype specific. Objectives: The development of recombinants capripoxvirus for protective immunization of ruminants against bluetongue virus (BTV) infection is described here. Methodology: Sheep (n=11) and goats (n=4) were immunized with bluetongue recombinant capripoxvirus (BTV-Cpox) expressing individually four different genes encoding two capsid proteins (VP2 and VP7) and two non-structural proteins (NS1, NS3) of Bluetongue virus serotype 2 (BTV-2). Results: Seroconversion was observed against NS3, VP7 and VP2 in both groups of animals. A specific BTV antigens lymphoproliferation observed in goats corroborates with a partial protection in sheep challenged with a virulent strain of BTV-2.

### **INTRODUCTION**

Since 1998, outbreaks of BT involving 6 distinct serotypes have occurred in the Mediterranean basin. The only BTV vaccines currently available are serotype specific Previous studies involving coexpression of VP2 and VP5 as virus like particles (VLPs) or in combination with core proteins confirmed the viability of a subunit BTV vaccination strategy. However, these vaccines have not yet been adopted for field use. A recent study

\*Contact author : E-mail: catherine.cetre-sossah@cirad.fr



using recombinant canarypox virus coexpressing genes encoding the VP2 and VP5 induced high level of protection in sheep but still remains serotype specific (Boone *et al.*, 2007). In contrast, expression of VP7 by a recombinant capripoxvirus provided partial protection of sheep against a virulent heterotypic BT virus challenge (Wade-Evans, 1996). In this study, recombinant capripoxviruses (BTV-Cpox), expressing VP2 or the conserved VP7, NS1 and NS3 proteins derived from BTV serotype 2 were developed in order to induce a heterotypic protection. These BTV-Cpox recombinants were administrated to goats and sheep and immune responses were evaluated in both species. Sheep were also challenged with a virulent strain of BT serotype 2.

## **CLONING OF BTV GENES AND GENERATION OF CAPRIPOXVIRUSES**

The four distinct full-length BTV genes NS1, NS3, VP7 and VP2 were cloned from a Corsican strain of BTV-2 into pKSCATpSGPT, a shuttle plasmid. All BTV genes were placed under the control of the early/late synthetic promote (PS) from vaccinia virus. The *Escherichia coli* xanthine-guanine phosphoribosyltransferase gene (*Ecogpt*), also under control of the PS promoter but in the opposite orientation, was used as a dominant selectable marker to isolate the recombinants (Boyle *et al.*, 1988; Falkner *et al.*, 1988). The insertion site in the capripoxvirus genome was the viral thymidine kinase (TK) gene as the site for insertion (Wallace, 2005). Final constructions were used to generate recombinant capripoxviruses by transfection of OA3Ts as described in Romero *et al.*, 1993.

## **IMMUNIZATION OF GOATS AND SHEEP**

Two groups of four Saanen goats and twenty-one Pré-Alpes sheep, purchased from a French region free of BTV infection were housed respectively in an insect proof stabling facility or a level 3 stabling facility. Animals were individually inoculated subcutaneously with either a suspension of  $2.10^6$  TCID<sub>50</sub> of an irrelevant recombinant capripox-HPPR vaccine expressing the haemagglutinin of the peste des petits ruminants virus (control group) or with  $2.10^6$  TCID<sub>50</sub> of each BTV-Cpox recombinant vaccine (BT-Cpox group). Three weeks after inoculation, all animals were challenged subcutaneously with either  $10^4$  TCID<sub>50</sub> of the BTV-2 attenuated vaccine strain for goats or virulent BTV-2 strain for sheep. Whole blood (EDTA and heparinised) was collected respectively

for RNA extraction and PBMCs isolation. Serum for antibody detection was collected immediately prior to inoculation and at weekly intervals. Sheep were observed daily after challenge for clinical manifestations which were eventually monitored, giving a final score for each animal.

## **IMMUNOGENICITY OF BTV-CPOX VACCINE IN GOATS AND SHEEP**

Antibodies to capripoxvirus as revealed by IFA were detected as early as 1 week post-vaccination in some animals and at 2 week post-vaccination in all animals (whether vaccinated with irrelevant HPPR-Cpox or BTV-Cpox). Three different ELISAs were used to analyze anti-NS3, anti-VP7 or anti-NS1 antibodies whereas VNT was used for anti-VP2 antibody detection. Goats and sheep gave both similar results. NS3 seroconversion was observed as early as 1 week post-immunization (PI) in the BTV-Cpox group compared to the control animals which only seroconverted at day 35, 2 weeks after the virulent challenge. VP7 seroconversion was observed in some animals inoculated with the BTV-Cpox recombinant at 3 weeks post immunisation (day 21) and for the whole group at 2 weeks post BTV challenge (day 35). The boost of anti-VP7 response at day 35 (Table 3) correlated with the anti-NS3 boost observed in the BTV-Cpox group animals. ODs of the BTV-Cpox group were significantly different from the values of the control group. Due to technique limitations, anti-NS1 antibody detection could not be interpreted. Anti-VP2 antibodies were detected by VNT in some animals vaccinated with BTV-Cpox group. All animals seroconverted at day 35. As expected, animals immunized with HPPR-Cpox seroconverted to HPPR whereas those immunized with BTV-Cpox did not.

## **CELL-MEDIATED IMMUNITY IN GOATS**

Antigen specific blastogenesis and lymphocyte proliferation were monitored at weekly intervals after the attenuated BTV challenge. A BTV specific proliferation was first observed at day 14 for some of the BTV-Cpox animals. A significant effect was observed for the whole group at day 28 compared to the control group. BTV specific blastogenesis correlated with the lympho-proliferation data at day 28. Specific lympho-proliferation to whole Cpox antigens was observed from day 28 to day 42 for the BTV-Cpox group and at day 42 only for control group animals. Similar results were obtained for blastogenesis.



## PARTIAL PROTECTION

The ability of BTV-Cpox vaccine to protect sheep and goats against disease and virus replication was evaluated respectively by scoring clinical signs after virulent BTV-2 challenge and by detection of BTV. One sheep out of eleven died at day 55 in the BTV-Cpox group whereas three animals out of ten died at days 53, 54 and 58 in the control group. The majority of animals presented clinical signs but a significant difference was observed between the two groups using the Kruskal-Wallis test with a  $p$  value  $<0.001$  (\*\*\*). BTV genome was detected in sheep blood at 4 and 7 days post-challenge. BTV genome was not detected in BTV-Cpox immunized goats but in control group.

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